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POTATO TUBER FORMATION IN THE SPACEFLIGHT ENVIRONMENT[1]

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Five potato (*Solanum tuberosum* L.) leaf cuttings were flown on STS-73 in late October, 1995 as part of the 16-day USML-2 mission. Preflight studies were conducted to study tuber growth, determine carbohydrate concentrations, and examine the developing starch grains within the tuber. In these tests, tubers attained a fresh weight of 1.4 g tuber^{-1} after 13 days. Tuber fresh mass was significantly correlated to tuber diameter. Greater than 60% of the tuber dry mass was starch and the starch grains varied in size from 2 to 40 μm in the long axis. For the flight experiment, cuttings were obtained from 7-week-old Norland potato plants, kept at 5°C for 12 h then planted into arcillite in the ASTROCULTURE™ flight hardware. The flight package was loaded on-board the orbiter 22 h prior to launch. During the mission, the flight hardware maintained an environment around the cuttings of 22 ± 2°C, 81 ± 7% RH, and a 12-h photoperiod using red and blue light-emitting diodes at a photosynthetic photon flux of 150 μmol m^{-2} s^{-1}. CO_2 concentration exceeded 4000 ppm during the dark period and was controlled during the light period to approximately 400 ppm. Video downlinking of images of the plants and CO_2 exchange data during the flight demonstrated plant vitality for the first 12 days of the mission followed by senescence of the leaves. The flight package was received 4 h after landing at the Kennedy Space Center and postflight processing of the samples was completed within 3 h. Four out of the five space-grown cuttings produced tubers that were similar in appearance and dimension to the ground control tubers. This is an important finding if potatoes are to be used as part of a bioregenerative life support system for long-term space exploration.

Potato Tuber Spaceflight Bioregenerative life support Microgravity

INTRODUCTION

White potatoes are a major source of food throughout the world. The National Aeronautics and Space Administration (NASA) is exploring the use of potatoes as one of the crops in a bioregenerative life support system that would utilize plants to recycle oxygen, carbon dioxide, and water as well as provide food. Potatoes have a high yield potential, a high ratio of edible to inedible biomass, and are easy to propogate and prepare for consumption (19). In addition, potato tubers are high in digestible starch and provide substantial amounts of protein (15).

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It is known that environmental conditions can affect tuber formation. For example, high temperatures inhibit tuber growth and lead to diminished starch content (11). As starch represents the major source of calories in potatoes, it is important to understand the factors that might regulate the amount of this carbohydrate in tubers in the spaceflight environment. A number of reports indicate that starch accumulation is reduced in plants exposed to spaceflight (2,4) or to ground-based microgravity conditions such as clinorotation (1,13). The reduction in starch concentrations might result from reduced photosynthesis in space-grown plants (16), perturbed translocation of carbohydrates throughout the plant (2), or altered enzyme activities in the starch synthetic or degradative pathways (1,10). However, existing information is limited on starch concentrations in plant storage organs such as tubers (10). Therefore, the usefulness of potatoes or other starch-storing plant tissues may be limited in a bioregenerative life support system for spaceflight or reduced gravity environments. The studies outlined in this article will allow us to determine if potato tubers are formed in the spaceflight environment, which will lead to studies of tuber starch metabolism and anatomy.

Several workers have shown that excised potato leaves can develop tubers from the axillary bud at the base of the leaf (5,6,14). These tubers accumulate large concentrations of starch. The deposition and structure of the starch in these developing tubers are essentially the same as in tubers developing on stolons on intact plants (3). Cell division and enlargement can occur within 24 and 96 h, respectively, after excision of the leaf from the mother plant. Therefore, excised potato leaves capable of forming tubers that fill with starch provide a compact model system for the study of tuber development and metabolism in space (17).

MATERIALS AND METHODS

Plant Propagation

Potato (*Solanum tuberosum* L. cv. Norland) plants, started from sterile-culture stem cuttings, were grown in a walk-in growth chamber at the Kennedy Space Center Space Biology Laboratory. Plants were grown in 1-liter plastic containers filled with peat/vermiculite and watered to excess four times daily with a complete nutrient solution (7). Chamber conditions were maintained at 18°C, 70% relative humidity, with a photoperiod of 12-h light:12-h dark. The lighting at the plant level was 150 μmol m^{-2} s^{-1} photosynthetic photon flux (PPF) and was supplied by cool white fluorescent lamps.

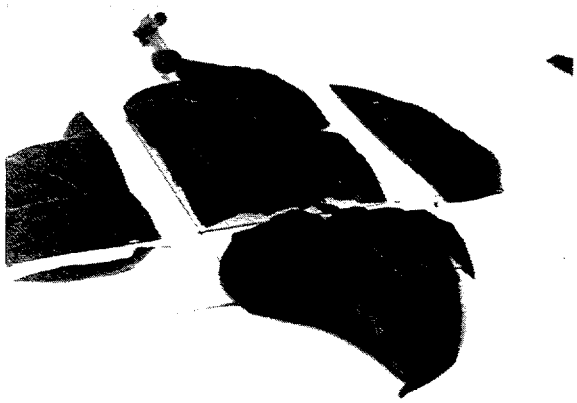

Figure 1. Potato leaf explants prior to insertion into the planting tray.

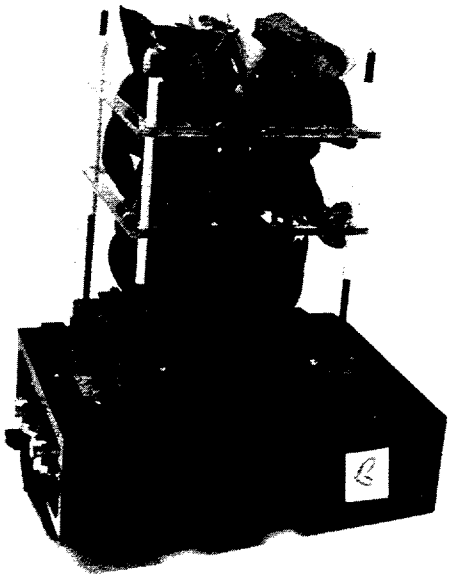

Figure 2. Five potato leaf explants inserted into the ASTROCULTURE™ planting tray.

Table 1. Environmental Control and Monitoring Capabilities of the ASTROCULTURE™ Flight Hardware

		16-Day STS-73 Mission			16-Day Ground Control		
Parameter	Set Point	Ave*	Max†	Min†	Ave*	Max†	Min†
Temp (°C)	21	22.1	23.9	21.2	22.0	23.9	20.6
Rel Hum (%)	80	81.3	88.0	76.6	78.2	81.4	75.5
CO_2 (ppm)	500	2308	4100	350	2102	5758	204

*Average values for the entire mission or ground control.
†Maximum and minimum for 12-h periods.

After 7 weeks, leaves were excised from selected plants for use in the fifth flight of the ASTROCULTURE™ program (12).

Preflight Preparation

Three uniform plants were selected to supply flight and ground control leaves. Leaf excision from the mother plants followed the method of Wheeler et al. (18). The leaves were taken 36 h prior to the scheduled launch from node positions 7 and 8 counting basipetally from the youngest leaf that was greater than 2 cm in length. A 1.5-cm portion of the stem was included as part of the explant and special care was taken to avoid disturbing the axillary bud. In order to fit within the flight hardware, excess laminar tissue was removed, providing a remaining leaflet area of ~135 cm^2 (Fig. 1).

Leaves were stored in a humid cooler at 5°C until 24 h prior to the scheduled launch. At that time the cut stumps were gently inserted into premoistened arcillite (calcined clay particles) in the rooting tray of the ASTROCULTURE™ flight unit. This unit provided plant lighting using red and blue light-emitting diodes, temperature and humidity control, and water delivery using a porous tube system (12). Additionally, the unit was configured for data collection, gas and fluid sampling, CO_2 control, camera observation of the plants, and an ethylene removal system. A cover, consisting of closed-cell foam with holes for the petioles, was placed over the planting tray. A total of five leaves fit within the planting tray (Fig. 2). The planting tray was then inserted into the growth chamber housing and the whole chamber assembly was integrated in the flight unit. The unit was transferred to the launch pad and loaded into the Space Shuttle at 22 h prior to launch. The STS-73 mission, a part of the United States Microgravity Laboratory-2, was launched from the Kennedy Space Center at 10:00 EDT on October 20, 1995. Environmental set points and actual conditions within the plant growth chamber during the mission and ground controls are found in Table 1.

Landing took place at the Kennedy Space Center at 06:45 EST on November 5, 1995 after a mission of 15 days and 20 h. The hardware was recovered 4 h after landing and deintegration of the planting tray commenced immediately. Plants were removed from the planting tray, photographed, measured, and frozen and/or fixed for later biochemical and/or anatomical measurements.

Mother plants for ground control leaves were started in the walk-in growth chamber at the Kennedy Space Center exactly 4 weeks after the start of the flight mother plants. Leaves were harvested from these plants at 7 weeks after planting and transported to Madison, WI for the ground control experiment. Ground control leaves were grown in the ASTROCULTURE™ hardware from November 17 through December 3, 1995. The hardware was maintained in a room of the University of Wisconsin Biotron under temperature conditions duplicating the middeck conditions of the STS-73 mission. Environmental data collected during the spaceflight mission were used to control the plant growth hardware during the ground control (Table 1). Plant harvest, measurement, and tissue preparation occurred exactly as during the postflight harvest.

RESULTS AND DISCUSSION

To ensure successful tuber formation in the conditions anticipated during the spaceflight mission, a number of preflight studies were conducted. These were ground-based studies using plants grown in a similar fashion to the ones described for the flight experiment.

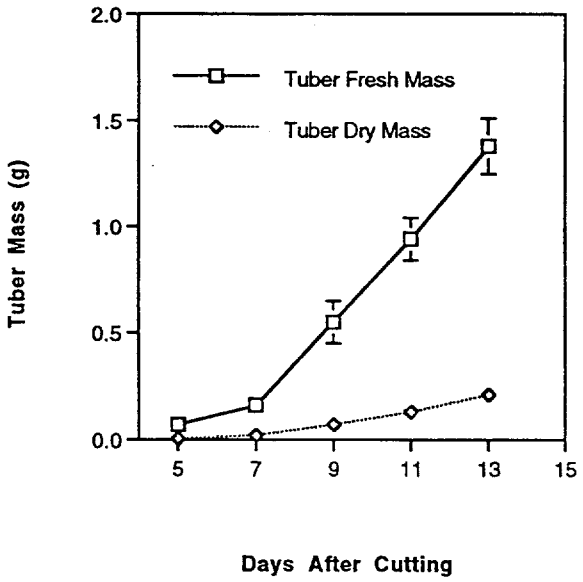

Figure 3. Growth of tubers in axils of potato leaf cuttings in preflight ground-based studies. Values represent the means of nine replicates and the standard error is shown.

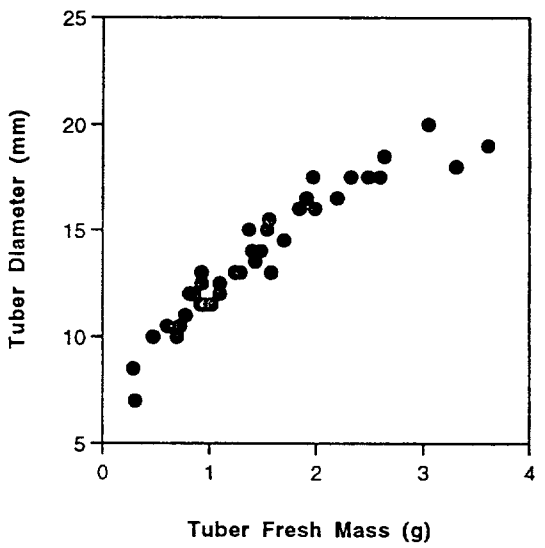

Figure 4. Fresh weight as a function of diameter in developing potato tubers in preflight ground-based studies.

Tuber Growth

Tuber size, measured as fresh mass or dry mass, typically increased in a linear fashion between 7 and 13 days after excision from the mother plants (Fig. 3). In all studies, tuber diameter was significantly correlated to tuber fresh mass (Fig. 4). These results indicated that adequate tuber formation and growth occurred within the time frame of the proposed flight experiment and that estimates of tuber mass could be acheived through nondestructive measurements of tuber diameter.

Tuber and Leaflet Carbohydrate Measurements

It was necessary to ascertain if the potato explants and tubers would contain starch and soluble carbohydrates of measurable quantities when cultured under conditions anticipated for the mission. Leaf cuttings were cultured in an arcillite rooting matrix and harvested after 14 days. Laminar, stem, and tuber tissues were frozen in liquid nitrogen, freeze-dried, and analyzed for starch, sucrose, glucose, and fructose as in Brown et al. (1). Quantifiable concentrations of these carbohydrates were present in all the tissue types (Table 2).

High levels of starch were present in tuber tissue with 8.5- and 70-fold lower concentrations in the stem and laminar tissue, respectively. Concentrations of the carbohydrate fractions were similar in the tissues of leaves from positions 5 through 8 counting basipetally from the apex of the mother plant (data not shown). These concentrations were easily measured and were comparable with previous studies (8). The extremely low concentration of fructose in the tuber tissue is consistent with the observation that fructose is a poor precursor for the synthesis of starch (9). These results indicated that measurable quantities of carbohydrates were present in all tissue types under the cultural conditions anticipated during spaceflight and that the choice of leaf position did not affect carbohydrate concentrations during subsequent tuber formation. Starch grains were

Table 2. Carbohydrate Concentrations in Laminar, Stem, and Tuber of 14-Day-Old Potato Leaf Explants in Preflight Ground-Based Studies

	Concentration [mg (g dry weight)$^{-1}$]		
	Laminar	Stem	Tuber
Starch	9 (2)	74 (12)	634 (34)
Sucrose	17 (3)	25 (2)	32 (9)
Glucose	28 (6)	34 (5)	3 (1)
Fructose	1 (1)	1 (1)	0 (0)

Values represent the mean of six replicates and the standard error is shown in parentheses.

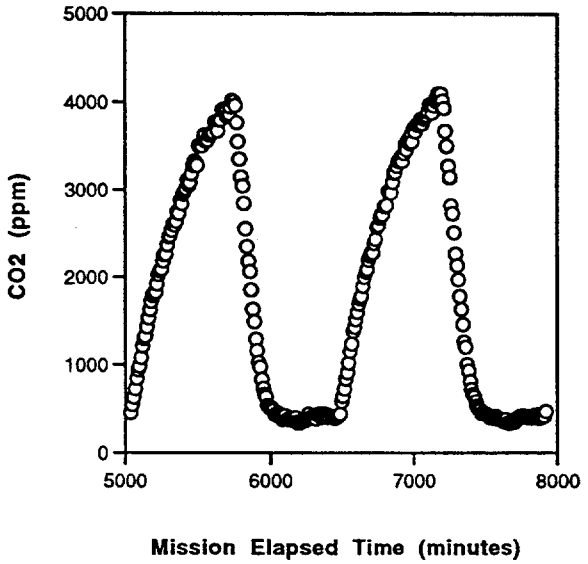

Figure 5. CO_2 concentration within the plant growth chamber from mission elapsed time 84 h (5040 min) to 132 h (7920 min).

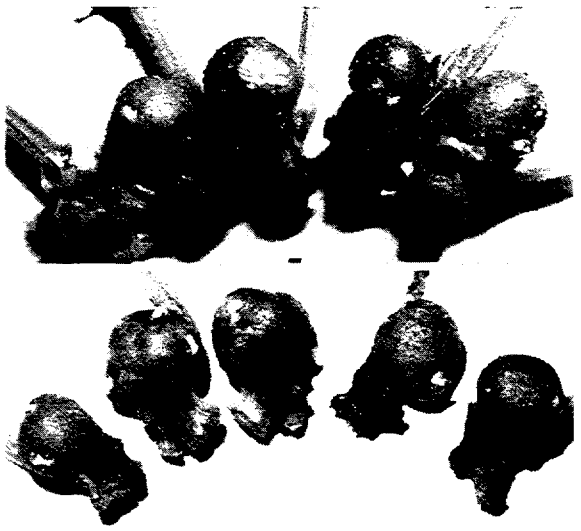

Figure 6. Potato tubers formed after 16 days in space (top) and ground control tubers (bottom). Scale is 1:1.

measured in tubers from 14-day-old plants. The grains ranged in size from 2 to 40 μm in the long axis. The smaller grains were spherical and the larger grains were eccentric.

In-Flight Measurements

During the STS-73 mission, concentrations of CO_2 in the growth chamber were periodically downlinked from the Space Shuttle to researchers on the ground. Measurements for one 48-h period are shown in Figure 5. The reduction in CO_2 during the light periods (5800–6520 min and 7240–7960 min) indicated that the plants were photosynthetically active during this period. This pattern was consistent until day 12 when there was a marked senescence of the leaves. These data, in combination with downlinked video images of the plants, indicated that the plants were healthy and fixing carbon for the majority of the mission.

Postflight Measurements

Tuber formation and growth took place in the spaceflight-grown tissue (Fig. 6, top). The size and appearance of the space-grown tubers were not significantly different from the ground control tubers (Fig. 6, bottom). Excluding the tuber on the far left in Figure 6 (top), the mean diameter of the space-grown tubers was not different from the ground control tubers, indicating the tuber fresh mass may not be significantly impacted by the space environment (Fig. 4). These results indicate that spaceflight is not an impediment to tuber formation. This is an important finding if potatoes are to be used as part of a bioregenerative life support system for long-term space exploration (19). The effects of the spaceflight environment on the composition and structure of starch and other components of the potato tuber will be defined upon completion of the biochemical and anatomical analyses.

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